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I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER OF PATENTS, WASHINGTON, DC 20231, ON THE DATE INDICATED BELOW.

BY: Pamela Wright DATE: August 31, 2001

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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of Darwin J. Prockop *et al.*	:	Group Art Unit: 1633
Appln. No.:	09/028,395	:	Examiner: Janet M. Kerr
Filed:	February 24, 1998	:	
For:	ISOLATED STROMAL CELLS FOR USE IN THE TREATMENT OF DISEASES OF THE CENTRAL NERVOUS SYSTEM	:	Attorney Docket **No. 9598-32US** (53844-5002-00) AUHS-318

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DECLARATION OF DARWIN J. PROCKOP PURSUANT TO 37 C.F.R. § 1.132

I hereby declare as follows:

1. I am the same Darwin J. Prockop who is a co-inventor, along with David G. Stokes and S. Ausim Azizi, of the invention described and claimed in the above-identified application.

2. Since filing this application, additional experiments have been performed, wherein I am an author of the reference publishing the results of said experiments, relating to use of marrow stromal cells (MSCs) for cell and gene therapy treatment of central nervous system (CNS) diseases, disorders and conditions.

3. I and co-inventor S. Ausim Azizi, have obtained data which demonstrate that recombinant MSCs engraft the mammalian CNS and provide therapeutic benefit in an art recognized model of CNS disease, *i.e.*, Parkinson's disease (PD). This data is disclosed in Schwarz et al., 1999, Human Gene Therapy 10:2539-2549, a copy of which is attached hereto as Exhibit "A."

4. Briefly, my colleagues and I, using the cells of the invention, further reduced the invention to practice in that rat MSCs were transduced using an retrovirus vector such that the cells express enzymes necessary for synthesis of L-DOPA, *i.e.*, tyrosine hydroxylase (TH, rate-limiting enzyme in dopamine synthesis) and GTP cyclohydrolase I (GC, enzyme necessary for synthesis of tetrahydrobiopter cofactor, BH_4, for TH). The transduced cells were engrafted into rats lesioned using 6-OHDA (6-hydroxydopamine), which is an art recognized model of human Parkinson's disease.

5. Donor cells were present in the recipient brains at 87 days post-engraftment and the cells had migrated extensively throughout the brain.

6. Although the proteins were expressed for 14 days due to transgene shutdown known to occur with this viral vector, the data demonstrated that L-DOPA and metabolites thereof were present in the engrafted brains (Exhibit "A", Figs. 3A-B), and the recipient rats demonstrated behavioral recovery (*e.g.*, decreased rate of apomorphine-induced rotation) (*id.* at page 2543, Fig. 2).

7. More recently, my laboratory has extended these observations and further reduced the invention to practice. The data are disclosed in Chapter 5 of the unpublished thesis of a doctoral candidate in my laboratory, Emily J. Schwarz, a copy of the pertinent pages of which, pages 102 to 121, is attached hereto as Exhibit "B."

8. More specifically, we have demonstrated transduction of rMSCs using self-inactivating retrovirus vectors expressing TH and GC downstream of PGK promoter (rMSC-pSIR-PGK-TIG). Further, using this art accepted murine model of PD, the data disclosed herein demonstrate behavioral correction (*e.g.*, decreased rate of apomorphine-induced rotation) compared with lesioned rats transplanted with control MSCs transduced with a non-specific (green fluorescence protein) GFP gene (Exhibit "B," page 109, Fig. 27).

9. Additionally, the data disclosed herein demonstrate production of L-DOPA and metabolites thereof in donor rat brains (Exhibit "B," page 111, Fig. 28), and that the MSCs preferentially migrated to damage sites and along needle tracks caused by the technique to produce the lesion (Exhibit "B," page 116, Fig. 31).

10. Moreover, the data disclosed herein demonstrate MSC differentiated into neurons (expressing neuronal marker NeuN, Exhibit "B," page 120, Fig. 33).

11. Thus, the data disclosed herein demonstrate that gene therapy whereby an exogenous nucleic acid encoding a beneficial protein is expressed in MSCs and the recombinant cells can be administered to a recipient to treat a disease, disorder or condition of the CNS in an art-recognized model of such disease, disorder or condition.

12. In addition, Horwitz et al., 1999, Nature Med. 5:309-313, which is attached hereto as Exhibit "C" and on which I am a co-author, have further reduced the invention to practice, demonstrating that cell therapy methods assessed in non-human animal studies using MSCs can be successfully applied to treatment of humans using MSCs.

13. More specifically, MSCs from normal HLA-identical or single-antigen-mismatched siblings were transplanted into children with severe deforming osteogenesis imperfecta (OI).

14. The data disclosed herein demonstrate that only as little as approximately 1.5-2% donor MSCs (determined by detecting Y chromosome in osteoblasts or DNA polymorphism in osteoblast of same-sex match) mediated clinically detectable improvement in various clinical osteogenesis parameters, including improved bone histology and mineral content (Exhibit "C", page 312, Figs. 3A-F); increased total body bone mineral content (*id.* page 312, Fig. 4B); fewer fractures; and achievement of normal median growth velocity (*id.* page 312, Fig. 4A).

15. These data demonstrate correlation between data obtained in murine OI model (Pereira et al., 1998, Proc. Natl. Acad. Sci. USA 95:1142-1147), and human *in vivo* data.

16. The observations of Horwitz et al., 1999 (Exhibit "C"), were extended in Horwitz et al., 2001, Blood 97:1227-1231, a copy of which is attached hereto as Exhibit "D" and which describes follow-up of the three OI patients for an additional 18-36 months beyond the original 6 month follow-up.

17. The data disclosed herein demonstrate that all three OI patients transplanted with normal donor MSCs in Horwitz et al., 1999 (Exhibit "C"), continued to demonstrate detectable improvement over controls in the clinical parameters assessed previously: increased growth depicted in terms of absolute bone growth (Exhibit "D", page 1229, Fig. 2A); increased growth rates which are similar to those of age-matched healthy children (*id.* page 1229, Fig.

2B); all three patients had slightly greater TBBMC (mineral content) than normal weight-matched children (*id.* at page 1229, Fig. 3A-B).

18. In sum, the human clinical data disclosed herein in Exhibit "D" demonstrate that, upon extended follow-up, MSC transplant recipients showed continued improvement. Further, the data establish that the effects of MSC-cell therapy are long lasting such that, *e.g.*, there is no problem with gene expression shutdown, and the like. Moreover, these data demonstrate that there is no detectable toxicity associated with MSC cell therapy.

19. In addition, the data disclosed in Chen et al., 2001, Stroke 32:1005, attached hereto as Exhibit "E", which was published after the instant application and followed the teachings of the specification as filed, demonstrate that MSC cell therapy, as taught by applicants, provides a therapeutic benefit in an art-recognized model of stroke. More specifically, in Chen et al., Dr. Chopp and his colleagues demonstrate that rats subjected to middle cerebral artery occlusion (MCAO), which is an art-recognized model for human stroke, demonstrated significant recovery of somatosensory behavior and Neurological Severity Score (NSS, which is a composite of motor, sensory, reflex, and balance tests) following transplantation with MSCs.

20. That is, $1x10^6$ (low-dose MSCs) or $3x10^6$ MSCs (high-dose MSCs) were injected into a rat tail vein either following or the in the absence of MCAO, and a battery of behavioral tests (*e.g.*, rotarod measurements, adhesive-removal somatosensory test, and NSS) were performed at various times after MCAO. The data disclosed herein demonstrate that rats treated using low-dose MSCs showed no significant difference from non-transplanted rats in these tests. However, rats treated using high-dose MSCs demonstrated significant improvement in the various tests compared to control rats whether the MSCs were administered at 1, 7, or 14 days after ischemia (Exhibit "E", Table 1 and Figs. 1 and 2).

21. The data disclosed herein further demonstrate that the donor MSCs were detected throughout the brain (*e.g.*, in multiple areas of the ipsilateral hemisphere, including cortexes, striatum of the ipsilateral hemisphere), with the vast majority in the ischemic core and its boundary zone and a few in the contralateral hemisphere (Exhibit "E", Table 2). These data are in accord with the observations of Schwarz et al. (Exhibit "C") that it appears that donor MSCs selectively migrate to the area of brain tissue damage.

22. Additionally, the data disclosed herein demonstrate that some donor MSCs were reactive for the neuronal markers NeuN (neuronal nuclear antigen) and MAP-2 (microtobule-associated protein 2) (Exhibit "E", Figs. 3 and 4), supporting that MSCs differentiate into neural cells in the brain.

23. These data further support that per the teachings of the specification as filed, cell therapy using MSCs can treat a CNS disease, disorder or condition, such as, but not limited to stroke/ischemia, as demonstrated in an art-recognized animal model of CNS disease, disorder, or condition.

24. Moreover, in Li et al., 2001, Neurology 56:1666-1672, a copy of which is attached hereto as Exhibit "F", Dr. Chopp and his colleagues further demonstrate that MSC cell therapy provides a therapeutic treatment for stroke in an art-recognized model of human stroke. Specifically, rats were subjected to MCAO followed by intracarotid arterial injection of $2x10^6$ MSCs. The rats were then assessed using nerologic functional tests (adhesive-removal somatosensory test and modified NSS), before and at 1, 7, and 14 days post-MCAO. Additionally, the rat brains were examined using histologic and immunohistochemical assessment.

25. Li et al., demonstrate that MSC delivered to the carotid artery distribute over an wide area of the ischemic core and penumbra (Exhibit "F", Fig. 1, and text discussing same). Further, rats treated using MSC cell therapy demonstrated significant improvement in adhesive-removal tests and in modified NSS (*id.* at Figure 2). Also, the data demonstrate that the donor MSCs were found throughout the recipient brain and expressed GFAP and MAP-2 in the ischemic ipsilateral hemisphere and only expressed GFAP in the contralateral hemisphere.

26. Therefore, the data disclosed in Li et al., further demonstrate that, following the teachings of the invention, MSC cell therapy can successfully treat a CNS disease, disorder or condition, *e.g.*, stroke, as demonstrated using an art-recognized animal model for human stroke.

27. Data disclosed in Olson et al. (2001, In: Tissue Engineering for Therapeutic Use, pp. 21-36, Ikada and Oshima, eds., Elsevier Science), attached hereto as Exhibit "G", on which I am a co-author, demonstrate that MSC cell therapy is useful for treatment of spinal cord injury (SCI) in an art-recognized animal model for human SCI.

28. Olson et al., demonstrate that MSCs grafted to the intact or injured rat spinal cord survive and express neuronal markers such as NeuN (Exhibit "G", page 31, lines 7-10).

29. Thus, in an art-recognized model of spinal cord injury, MSC therapy has been demonstrated to provide a therapeutic benefit in treating this CNS disease, disorder or condition.

30. Further, in Chopp et al. (2000, NeuroReport 11:3001-3005), a copy of which is attached hereto as Exhibit "H", MSCs were transplanted into the spinal cord in a rat model of spinal cord injury.

31. The data disclosed in Exhibit "H" demonstrate that MSCs injected into the rat spinal cord 1 week after a weight driven implant injury provide significant improvement in functional outcome in transplanted animals using functional outcome measurements with the Basso-Beattie-Bresnehan (BBB) score, which is an art-recognized method of assessing SCI. The data demonstrate that rats injected with 2.5×10^5 MSCs at the epicenter of injury exhibited steady recovery that had not plateaued at five weeks and had significant improvement on BBB scores (*id.* at page 3003, Fig. 1).

32. Further, Chopp et al., demonstrate that donor MSCs are present in the spinal cord and express neural protein markers (*e.g.*, NeuN) (*id.*, at page 3004, Fig. 2).

33. In summary, these data presented herein demonstrate that pursuant to the teachings of the specification as filed, the use of MSCs has been further reduced to practice. That is, the data described herein amply support that MSC cell and/or gene therapy in CNS diseases, disorders or conditions has been reduced to practice in art-recognized models of human CNS diseases. More specifically, the data discussed herein demonstrate treatment of Parkinson's disease, stroke, and spinal cord injury according to the methods disclosed in the specification as filed. Therefore, the data described herein demonstrate that a method for treating those diseases according to the methods disclosed in the specification as filed, is clearly enabled under 35 U.S.C. §112, first paragraph.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date August 28, 2001

Darwin J. Prockop

Darwin J. Prockop, M.D., Ph.D.